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Rabies virus infection: Role of the rabies virus phosphoprotein in producing neuronal injury mediated by mitochondrial dysfunction and oxidative stress

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Background: Our previous work in a mouse model of experimental rabies showed neuronal process degeneration in association with severe clinical disease. Cultured adult rodent dorsal root ganglion neurons infected with the challenge virus standard-11 (CVS) strain of rabies virus (RABV) showed axonal swellings and reduced axonal growth with evidence of oxidative stress. We have shown that CVS infection results in increased reactive oxygen species (ROS) production and mitochondrial Complex I activity. The RABV phosphoprotein (P) was detected by immunoblotting in RABV-infected purified mitochondrial extracts from mouse neuroblastoma cells and in Complex I immunoprecipitates from the extracts. A plasmid expressing P in cells increased Complex I activity and increased ROS generation, whereas expression of other RABV proteins did not. Expression of a peptide from amino acid 139-172 of P increased Complex I activity and ROS generation similar to expression of the entire P protein, whereas peptides that did not contain this region did not.

Methods & Materials: Mutational analyses were performed to evaluate the role of the RABV P in Complex I activity and ROS generation.

Results: We performed alanine mutagenesis of overlapping triplicate adjacent sites over the 139 - 172 P region and on seven conserved amino acids and mutagenesis to both alanine and aspartate on four serine residues in this region. Mutational analysis suggests importance of the 145-151 and 157 to 169 regions of P. Three (159, 162, 166) of four serine residues were important and double alanine mutations had greater effects on activities. Six (144, 146, 147, 155, 167, 170) of seven conserved amino acids were also important.

Conclusion: A region of the RABV P interacts with Complex I in mitochondria causing mitochondrial dysfunction, increased generation of ROS, and oxidative stress. Therefore the RABV P plays a key role in the induction of mitochondrial dysfunction and generation of ROS resulting in oxidative stress in rabies virus infection through an interaction with Complex I. The resulting mitochondrial dysfunction produces oxidative stress in neurons that causes acute degenerative changes affecting neuronal processes resulting in a severe and fatal clinical disease. This information will be important for the future development of novel therapies for rabies.

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Dengue 2 virus infection associated vascular endothelial cellular stress response imaged by high resolution electron and correlative microscopy shows distinct evidence of altered cytoskeleton and vesicular traffic

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Background: Dengue virus (DENV) is an enveloped *Flavivirus* that is of significant global public health importance. The virus is transmitted through bite of infected *Aedes egyptii* mosquitoes and can cause cinical disease in humans ranging from mild asymptomatic subclinical infections to severe life threatening forms called dengue hemorrhagic fever and shock syndrome (DHF/DSS). The pathogenesis of dengue virus infection remains incompletely understood. Endothelial cells have been shown to be natural targets of dengue viruses and dysfunctional responses of these cells cause the severe hematological crisis of DHF. The nature of cellular stress of the vascular endothelium to DENV remains unknown. In the present study we used a correlative integrated high resolution microscopy approach (CLEM) to image the nature of fine structural changes in DENV 2 infected endothelial cells of hepatic sinusoidal origin.

Methods & Materials: DENV 2 infected cells were observed through early and late time point scales and virus replication detected through immunofluorescence and polymerase chain reaction assays. Infected cells were harvested and observed in three different microscopy platforms for high resolution imaging. Atomic Force Microscopy on live cells, confocal microscopy for tracking viral antigens and cryosubstituted cells imaged under high resolution transmission electron microscopy and tomography for fine structural changes.

Results: The integrated CLEM observations revealed early changes in vesicular traffic; actin depolymerization; autophagic response; cisternal dilatation of the endoplasmic reticulum and changes in cell surface caveolar morphology. Moreover, a very unique observation was co-localization of virus with cellular mitochondria but absence of apoptotic morphology.

Conclusion: Taken together, these findings, for the first time provides insight into fine structure of endothelial cellular stress response suggesting that basic physiological alteration in actin dynamics and signaling pathways are fundamental in endothelial dysfuction directly affected by the virus. Elucidation of this host pathway has potential for developing novel drug targets.

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